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Novel Breast Cancer Brain Metastasis Patient-Derived Orthotopic Xenograft Model for Preclinical Studies

（ヒト患者由来移植片を用いた前臨床動物モデルとしての新規乳癌脳転移モデルの確立と癌微小環境の検討）

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1. Abstract

Despite recent remarkable advance in diagnosis and treatment, more than 40,000 patients die from breast cancer annually in the United States alone (Siegel et al., 2019). Majority of death is due to cancer recurrence and distant metastasis, where brain metastasis is the deadliest with a dismal 20% one-year survival (Kirsch et al., 2005). One of the reasons for this poor outcome is because of lack of effective treatment option other than radiotherapy, which is also not effective unless the lesion is localized. The development of novel therapeutics for breast cancer brain metastasis have been hampered by the lack of preclinical models that reliably reproduce clinical characteristics of human brain metastasis (Johnson et al., 2001). Commonly used rodent brain metastasis model is injection of cancer cells into the heart or carotid artery; however, this technique generates a scattered cluster of cancer cells rather than tumor mass that is morphologically very different from human metastatic brain tumor (Hausser et al., 2005). The patient-derived xenograft (PDX) model is a transplantation of human tumor tissue into the same (orthotopic) or different (ectopic) organ of immunodeficient mice. PDXs retain genomic, transcriptional and phenotypic features of the original tumor even after long-term continuous passage in vivo, and they are reported to relatively closely replicate drug response of a human tumor compared with syngeneic models (Mukohyama et al., 2016). Our group and the others demonstrated that cancer biology of orthotopic PDXs is closer to human tumor than subcutaneous PDXs (Okano et al., 2020); however, there was no established breast cancer brain metastasis PDX with high survival that provide stable results. In this thesis, I report the establishment of a novel orthotopic breast cancer brain metastasis PDX model that better mimics human cancer than the commonly used ectopic PDX, as well as further studies on the pursuit of clinical relevance of basic research findings.

2. Materials & Methods

I used breast cancer brain metastatic tumor resected from January 2017 to December 2019 at the Roswell Park Comprehensive Cancer Center to establishment of the PDXs. One mm^3 fragments of metastatic brain tumors of breast cancer patients were xenografted in brain caudate putamen or MFP of immunodeficient NSG mice using various methods.

3. Results

I tested 3 methods for tumor implantation in brain: direct placement of tumor fragment using fine forceps (Forceps method), and injection of gently crushed tumor fragment using beveled 23 G needle with syringe (Needle method) or a pipettor with 10-ul pipette tip of 1 mm bore (Pipette method). PDXs in 8-10 mice were generated using each method. Post-operative mortality was zero with the Forceps and Pipette methods. However, 5 of 8 mice died within a day of implantation with the Needle method. At 6 weeks post-implantation, tumors were detectable in 80%, 67%, and 100% of surviving mice implanted using the Forceps, Needle, and Pipette methods, respectively. Tumor volumes were less variable with the Pipette compared to the Forceps method. Unlike the 100% engraftment for brain PDXs, the engraftment rate of patient brain metastasis implanted ectopically in MFP was only 75%. However, both orthotopic and ectopic PDXs grew about 2-fold faster after 3x serial passaging (all t test $p < 0.05$). To assess the effect of tumor site on drug sensitivity, I compared response of the brain and MFP PDXs ($n = 5$ each) to one intravenous dose of Epothilone B, a taxane-like drug that can pass through the blood-brain barrier. While this drug is effective against primary breast tumors, it failed to demonstrate activity against breast cancer brain metastasis tumors in a recent phase II clinical trial. Tumor progression of MFP PDXs was retarded by approximately 3-fold compared to control mice ($p < 0.05$). On the other hand, the drug had no effect on brain PDXs ($p = 0.95$). Our result implicate that the use of our model could have detected the ineffectiveness of this compound prior to clinical trial.

4. Discussion

I have established a novel orthotopic breast cancer brain metastasis PDX model that better

mimics human cancer than the commonly used ectopic PDX. The advantage of our method is that only one cubic mm of tumor was sufficient for engraftment, and tumor growth was observed within almost all mice brains at one month after transplantation. This is important because it demonstrates that our method can be used to generate PDXs relatively quickly without wasting precious human specimens. Furthermore, with our method, passage number can be reduced by transplanting human specimens directly into the mouse brain with a high engraftment rate. In contrast to other methods, the pipette tip method I have developed has not only low postoperative mortality and high engraftment rates, but also provides more accurate positioning and smooth distribution of tumors during the transplant procedure. These are important factors as a preclinical model for patients with brain metastases who have not many times. Many studies have shown the usefulness of embedding tumors in Matrigel for tumor implantation. However, in the current study I observed that the growth of brain PDXs was significantly retarded by Matrigel compared to PBS. I could not find a previous citation of such an observation in the existing literature. The effect that I have observed could be because Matrigel inhibits tumor growth factors in the brain environment. As PDX tumors grow, their human tumor stroma is replaced by mouse stroma (Kluin et al., 2018). In our comparison of transcriptomes of original brain metastasis from one patient and its xenografts in mouse brain and MFP, I observed that approximately 15% of RNA transcripts of PDXs were murine. As expected from the loss of human stromal cells, overall human gene expression in both PDXs was significantly different from the patient’s tumor. These results show also that the tumor microenvironment can affect gene expression in cancer cells of the tumor. To investigate the drug response in this study, I used epothilone B, which has a high anti-cancer activity against primary breast cancer tumors and can cross the brain-blood barrier, however it failed a phase 2 clinical trial for effectiveness against breast cancer brain metastasis. In our study, while brain metastasis PDXs grown ectopically in mouse MFPs responded to the drug, with tumor growth retarded by about a third compared to untreated tumors, orthotopic PDXs did not respond to treatment. This results suggest that our newly established novel breast cancer brain metastasis PDX model mimicked the clinical trial result, thus, I can expect that our model can appropriately prevent ineffective compounds such as Epothilone B from entering the clinical trial and expose patients with unnecessary side effects when our model is used in preclinical studies.

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Publication list

Ⅰ Thesis article

Novel Breast Cancer Brain Metastasis Patient-Derived Orthotopic Xenograft Model for Preclinical Studies

Oshi, M., Okano, M., Maiti, A., Rashid, O., Saito, K., Kono, K., Matsuyama, R., Endo, I., and Takabe, K.

Cancers. Vol.12, No.2, 444, 2020

Ⅱ Thesis associated articles

1. G2M Cell Cycle Pathway Score as a Prognostic Biomarker of Metastasis in Estrogen Receptor (ER)-Positive Breast Cancer

   Oshi, M., Takahashi, H., Tokumaru, Y., Yan, L., Rashid, O., Matsuyama, R., Endo, I., Takabe, K.

   International journal of molecular sciences Vol.21, No.8, 2921, 2020
2. A Novel 4-Gene Score to Predict Survival, Distant Metastasis and Response to Neoadjuvant Therapy in Breast Cancer

   Oshi, M., Katsuta, E., Yan, L., Ebos, J., Rashid, O., Matsuyama, R., Endo, I., Takabe, K.

   Cancers Vol.12, No.5, 1148, 2020
3. The E2F Pathway Score as a Predictive Biomarker of Response to Neoadjuvant Therapy in ER+/HER2- Breast Cancer

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   Cells Vol.9, No.7, 1643, 2020
4. Intra-Tumoral Angiogenesis Is Associated with Inflammation, Immune Reaction and Metastatic Recurrence in Breast Cancer

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5. CD8 T Cell Score as a Prognostic Biomarker for Triple Negative Breast Cancer

   Oshi, M., Asaoka, M., Tokumaru, Y., Yan, L., Matsuyama, R., Ishikawa, T., Endo, I., Takabe, K.

International journal of molecular sciences Vol.21, No.18, 6968, 2020

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